SOME ECOLOGICAL ADAPTATIONS OF CERTAIN FERN PROTHALLIA—CAMPTOSORUS RHIZOPHYLLUS LINK., ASPLENIUM PLATYNEURON OAKES.

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The part played by the prothallia in the ecological history of ferns has been given but little attention. If the studies on apogamy and related phenomena be left out of consideration the work along this line is very limited indeed. What work has been done may be arranged in three groups, as it has had to do with the increase of growing points by branching of the prothallia or by the production of special proliferations, the influence of light upon the germination of the spore and the development of the prothallium, the influence upon the prothallia of variations of water supply.

(*A*) Goebel (II: 197–210) has summed up the findings concerning the vegetative increase of the prothallia of homosporous ferns. Briefly stated there are two general types of growth, that which leads to the formation of a more or less regular heart-shaped plant, and that which leads regularly to considerably branched forms. Of the second group the *Vittariaceae* and *Hymenophyllum* show a lobular or ribbon-like body which arises by the branching of a simple primary cell plate. In *Trichomanes*, also of the second group, the prothallium is a mass of more or less branched filaments. With reference to those forms which regularly develop heartshaped prothallia Goebel (II: 203) says: "Young fern plants which have not yet formed typical meristem easily pass over again into the filamentous stage in feeble illumination. . . . In older prothallia this only takes place if they have lost their meristem and are enfeebled by unfavorable environment. Commonly these conditions result in the production of pluricellular shoots." To this last group mentioned by Goebel belong the plants often cited as producing adventitious outgrowths following mutilation in hybridization experiments. This author also mentions a case of proliferation by adventitious shoots from the base of an old prothallium of *Osmunda regalis* (loc. cit. I: 49). More recently several authors have found

that under peculiar culture conditions the prothallia of many ferns will produce special proliferous buds or gemmae.

In the present paper there will be shown yet another type of vegetative increase, and since the plants under consideration show no tendency to form special proliferous branches or gemmae, the literature dealing with that phase of the subject will be passed with the above brief mention.

(*B*) It has long been held generally that abundant moisture is necessary for the germination of spores and the normal development of the prothallia of homosporous ferns. In fact the ability of any such structures to live without a considerable constant supply of water has but very recently been shown. Goebel in the work referred to above (II: 426) describes special tuberous outgrowths produced by the prothallium of *Anogramme chaerophylla*, which covered with soil, may survive dry seasons and continue growth upon the return of favorable conditions. Little attempt has been made to test the ability of normally developed prothallia to survive extended periods of drought. Campbell (p. 85) cites a case of prothallia of *Gymnogramme triangularis* which were found growing after the dry season in the neighborhood of Stanford, Cal. He also states that the plants of a culture survived exposure to dry air in the laboratory during a whole summer. The results of the first published attempt to determine the extent to which fern prothallia could survive conditions of extreme desiccation appeared in an article by the present writer in 1913 (Pickett, 1913). Discrepancies in the results of that set of experiments, and the possibility of broadening the scope of the work to include other species have led to the present study.

(*C*) The influence of light on the germination of fern spores and the development of prothallia has been the subject of more investigation than both the topics just mentioned. All investigators agree as to the necessity of light for normal complete germination, and to the tendency of plants to develop attenuated or filamentous forms when in reduced light. The present work is concerned primarily with the effect of variation in light intensity under otherwise constant conditions.

This paper embodies the results of attempts to determine the ability of the prothallia of two ferns, grown under control, to survive exposure to conditions of extreme desiccation, to extreme drought conditions as found in nature, and to extremes of temperature as

found in nature; to determine the effect of variation of light intensity upon development, and finally by checking up results from controlled cultures with findings in the field, to determine whether or not the peculiarities found are developed or of use in nature.

CAMPTOSORUS RHIZOPHYLLUS Link.

As previously reported (Pickett, 1913, pp. 643–644), prothallia of this fern may survive an exposure of six weeks to air dried by passing through glycerine, but are killed by an exposure of four to six days over sulphuric acid in a closed desiccator. There are two possible explanations of this great variation, either the air is not entirely dried by the passage through the glycerine, or injurious gases are given off by the sulphuric acid. The work of Schröder and of Irmscher on mosses, as well as check experiments performed by the writer, seem to eliminate entirely the possibility of injurious gases being given off by the sulphuric acid. To determine the thoroughness of desiccation the former experiments have been repeated and new apparatus planned to furnish more thoroughly dried air.

In the writer's earlier experiments, as a result of reduced pressure secured by means of an aspirator, a current of air was made to pass through two 20 cm. U-tubes containing glycerine and crumpled filter paper saturated with glycerine, and then through small vials containing the material to be desiccated. In this apparatus air bubbles of from 2–3 cc. required 5–10 sec. in passing through the glycerine in the bottom of the tubes. The air was in further contact with the saturated filter paper about 15 min. In the experiments now to be described five methods of removing the moisture from the air have been employed, designated I, II, III, IV, V. To secure pressure, positive or negative, for the production of a constant stream of air the apparatus shown in text-fig. 1 was used. Water from the tap allowed to flow through *W* into the funnel in a stream about 1/3 as large as the bore of the tube *B* (4 mm.) in passing intermittently through the reverse curve at *B* carries bubbles of air with it into the aspirator jar *C*. The continuous stream of air and water passing into *C* tends to force the air through the tube *V* to *E* and thence through the other pieces of apparatus. Limiting the flow of air by means of screw cocks at *I* causes an increase of pressure in *C* until first the water and then the surplus air is forced out through *D*. The heights of tubes *D* and *B* determine the pressure available, and the number of tubes

(*B*) leading into *C* determines the volume of air available. In the present work $D = 1$ m., $B = 2.5$ m., and two of the latter were used to keep three sets of apparatus running. Tubes of the number

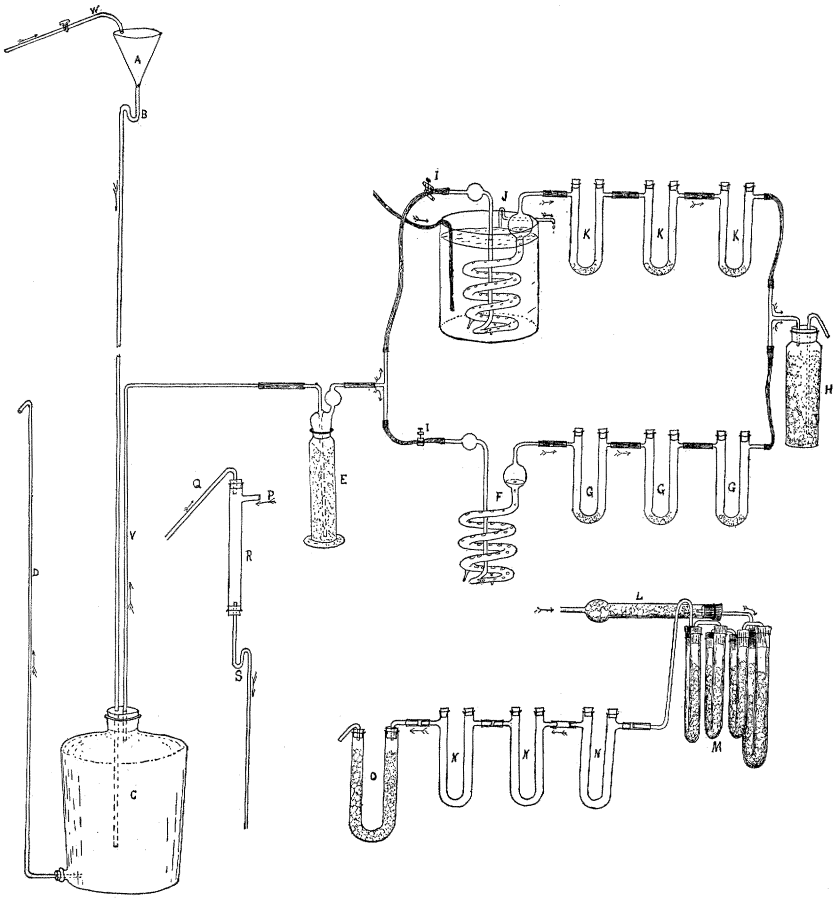


FIG. 1. Desiccation apparatus. *ABCD*, pump for securing positive air-pressure. *W*, inlet from water main. *P–S*, pump to furnish negative pressure. *E*, first drier filled with filter paper saturated with glycerine. *H*, *O*, checks filled with fused calcium chloride. *F–G*, glycerine desiccator and tubes. *I–K*, sulphuric acid desiccator and tubes with cooling jar about Winkler's tube. *L–O*, calcium chloride desiccator and tubes. (× 1/12.)

and dimensions given furnish abundant pressure and volume for easy control. Negative pressure was secured by using the pump *P–S*

instead of the funnel tube *A–B* and allowing the water and air to flow from the free end of the tube below. Water passing slowly from the tap through *Q* into the chamber *R* carries air through the curve at *S* and reduces the pressure in *R* so that a current is forced through *P* by external atmospheric pressure. When *P* is connected with the delivery tubes of the other pieces of apparatus the air current passes through them as before. Control of the air current is secured by screw cocks as above. The actual difference of pressure in the desiccating tubes with the different methods of securing pressure is slight, and as results have shown, is wholly negligible for the work in hand.

I. A glycerine desiccator was so arranged (text-fig. 1) that a current of air passed first through a wash bottle, *E*, filled with crumpled filter paper saturated with glycerine, and then as bubbles of approximately .1 cc. capacity, through a column of pure glycerine 1 m. long in a Winkler's potash tube, *F*. The passage of a bubble through this tube required 10–12 min., the time variation being due to variations of temperature and the resulting difference in the viscosity of the glycerine. The dry air next passed through 12 cm. tubulated drying tubes, *G*, and finally out through a 250 cm. wash bottle, *H*, filled with fused calcium chloride. This last element was used to prevent any possible entrance of moisture by diffusion or by currents resulting from changes in temperature. The clumps of plants and soil were carefully removed from the culture saucers when the soil surface began to show dry, and placed in the drying tubes, *G*. They were thus directly in the path of the current of dry air.

II. The second apparatus was an exact duplicate of that described under I, but the Winkler's tube contained a 1 m. column of c.p. sulphuric acid, sp. g. 1.84. The air bubbles, of about .03 cc. capacity, passed through the acid in a little less than 1 min. In check experiments the presence of injurious gases was guarded against by placing the tube, *J*, in a jar, as shown, through which a continual stream of water passed, keeping the temperature of *J* at from 13° to 15° C.

III. A third set of apparatus was so arranged that the current of air passed through a 100 cc. calcium chloride tube, *L*, and then through four U-tubes, *M*, all filled with medium size lumps of fused calcium chloride. This series of tubes was equivalent to a 1 m. column of the chloride. From the tubes the dry air passed over the specimens in the drying tubes, *N*, and out through the final check tube, *O*, filled with the chloride. To insure continual drying of the air the

chloride tube, *L*, was so connected that at the beginning of deliquescence of its contents it could be replaced by a fresh tube. A current of carbon dioxide was made to pass through this apparatus for 2 hrs. and then a current of air from the pressure apparatus for 18–24 hrs. before the specimens were placed in the drying tubes. In use the rate of air passage was slightly more rapid than in I.

IV–V. As checks on the experiments in which air currents were used two Fresenius closed desiccators were used, one (IV) with a 2 cm. layer of fused calcium chloride in the bottom and the other (V) filled 1 cm. deep with c.p. sulphuric acid. Parallel check experiments with soil and green tissue show the desiccation in the air currents and in the closed desiccators to be the same. Because of the slow passage of air in I and II, but small quantities of material will be dried quickly.

C. rhizophyllus, Extreme Desiccation.—Portions of soil of about .25 cc. volume, bearing well-developed prothallia with mature antheridia and archegonia were removed from the culture saucers to the drying tube as noted above. Several such clumps of soil and plants were placed in a tube at one time so that portions could be removed each day as wanted, with a minimum danger of mutilating the plants. For recovery the desiccated soil and plants were placed lightly on moist sterile soil under a bell jar in full diffused light in the greenhouse. In all cases recovery or survival has been taken to mean the ability to continue growth or to produce living sperms. The averages of results of experiments with the various methods are given below. The time in days is reckoned from the time when plants were put into the drying tube up to that of removing a clump which failed to show a living plant. The variation did not exceed one day in any case of duplicate experiments. The clumps were removed for revival at 8 A. M. each day. No attempt was made to determine the exact number of hours required for fatal exposure. Individual differences of plants would make such work extremely difficult; and the results would in the end be but averages for the species, such as we have by the method employed.

Results.— I. Glycerine desiccator.....all plants dead on the 7th day.
II. Sulphuric acid desiccator..... " " " " " 7th "
III. Calcium chloride tube..... " " " " " 6th "
IV. Fresenius, calcium chloride..... " " " " " 6th "
V. Fresenius, sulphuric acid..... " " " " " 6th "

These results agree very closely with those secured with the closed desiccators as formerly reported (Pickett, '13), the slight discrepancy being accounted for by the use of larger and older plants in the present work. They also clear up the discrepancy between the results with the glycerine desiccator and the sulphuric acid desiccator as previously reported, and give an important check to the value of the three agents used in drying currents of air. While not quite as striking as earlier results, in view of the extreme conditions, they are worthy of consideration. This becomes more evident if the data be compared with the following from non-resistant forms. Prothallia of *Onoclea struthiopteris*, *Dryopteris stipularis* and *D. mollis* used in check experiments have been killed by allowing soil of cultures to be dry for 2 days in sunlight, and can withstand but 2–3 hours exposure in the desiccator.

It may well be noted here that several times small plants, bearing 10–15 leaves, of *Polytrichum piliferum* were placed in the drying tube with the fern prothallia and recovered completely although left 3–5 days after the prothallia were dead. Small plants of an unknown *Bryum* survived similar exposure without damage. This is taken to show that injurious gases were not present in harmful quantities, if at all.

Cultures for experimental work were regularly made by sowing free spores and crushed sporangia on rich loam in 12 cm. unglazed clay saucers with perforated bottoms. These saucers were placed in others 18 cm. in diameter. Irrigation was controlled by supplying water as needed to the outer saucer. Both saucers and soil were sterilized 1.5–2 hrs. at a temperature of 130° C. in an autoclave each day for three days before the spores were sown. The cultures were covered with tall bell jars with one side raised by a cork 1 cm. high to provide ventilation. As has been already reported (Pickett, 1914) cultures of this fern do not show uniform development. Some spores germinate in ten days or less, and by rapid growth produce sexually mature plants in ten to twelve weeks. Other plants develop slowly, and some spores lie dormant for three to four months under conditions suitable for germination. Numerous cultures have been made from spores of fronds collected in the field in March and April, a clear proof that some spores even live through the winter.

Extreme Natural Conditions, Experimental.—Cultures of mature plants were exposed to long dry periods in full sunlight and in full diffused light in the greenhouse, the conditions being approximately

those found in nature in the growing season. Diffused light was secured by means of a muslin screen 2 m. above the culture tables or by means of a double layer of filter paper pasted to the bell jars and half covering them. As already found, the exposure of air-dry cultures to full sunlight during periods of three to five weeks proves fatal. Cultures exposed for nine weeks to normal dry air without water but shielded from the direct sunlight showed a few living plants. One of the cultures has been subjected to periods of drought one to two weeks in length during a total period of sixteen months, and has but rarely been watered twice in succession without an intervening dry period. Occasionally it has been flooded, and has shown a new crop of sporophytes after each flooding. 25 per cent of the plants of this culture were living at the end of the sixteen months period, although they had undergone changes, as will be noted later.

On December 8, 1915, soil cultures with mature prothallia were placed outside the greenhouse, protected from direct sunlight by the building and an adjoining wall, and covered by bell jars. On March 26, 1914, cultures returned to the greenhouse showed all plants dead or injured. Injured plants had much the appearance of plants injured by too long exposure to drought, showing old dead tissue and groups of younger active marginal or meristematic cells. The lowest temperature to which these cultures were exposed was − 12° C. in this protected location, although temperatures around − 6° C. prevailed through January and February.

Field Notes.—A brief review of the weather conditions during the summer of 1913 will give the field notes their full value. The summer was extremely dry and warm in southern Indiana. Following the flood period of March 23–27 with 9.2 in. of rain, April was much drier than usual. Then a period of 146 days, from April 30 to September 12, showed but 35 showery days, with a total precipitation of but 6.3 in. Between Sept. 13 and 30 there was a rainfall of 2.78 in., and then the drought continued up to Oct. 17, with but .48 in. in the interim. During the same period the following temperature conditions were recorded:

	Mean Maximum	Maximum
May	25.5° C.	35.° C.
June	28.2	40.
July	34.4	41.9
August	33.2	38.3
Sept. 1–12	35.	38.8

This is the official record for the U. S. Weather Bureau station at Bloomington, Ind., and the figures show the readings of standard sheltered instruments. When placed in direct sunlight without reflecting surfaces and in freely moving air, the instruments showed a maximum of 54.4° C. These conditions were disastrous for many forms of vegetation. Midsummer and late annuals were killed outright. Ferns, except the early fruiting species or those growing in most favorable localities, failed to mature spores. Even the hardier soil-growing mosses showed remarkable mortality.

Camptosorus patches were visited in October and December, 1913, and in early March and April, 1914. In more exposed places, on limestone ledges, but few spores were matured in 1913, and most of the sporophytes suffered, some being killed by the dry summer. No prothallia were found in these places up to April, 1914, although they may usually be found in the autumn. On April 16, careful search was made in a wide wooded ravine where *Camptosorus* is abundant on large blocks and fragments of limestone which are matted with mosses, especially *Anomodon attenuatus* and *Brachythecium oxycladon*. Because of the numerous springs in this ravine, the drought was not as severe as in more exposed locations. Almost a full crop of spores had been produced in 1913, and on the date given, a few prothallia were found on soil in pockets protected by the mat of moss. Some of these prothallia showed sporophytes. A few were quite large and showed marginal outgrowths like those found on old prothallia of cultures, to be described later. Of course all these prothallia must have survived the winter, as might be expected with the protection of such a location. The largest plants were very much like those of sixteen months old greenhouse cultures, and very probably had endured the tempered drought of the summer of 1913. Fronds collected at this place on March 31 have furnished an abundance of readily germinating spores. Usually prothallia are easily found about colonies of *Camptosorus* from late August to cold weather, and again after April, although they do not survive winter in exposed situations in this region. It would be of great interest to the writer to know how far north they have been found regularly surviving the winter.

Proliferation or branching of fern prothallia is by no means unknown. Some forms produce normally a branched protonema-like form. Some have been described as producing hair-like outgrowths from marginal cells under conditions of special stimulus. Other forms

produce proliferations—sometimes mistaken for apogamous sporophytes—from unusually active cells of the surface. As described by Goebel (I: 49), prothallia of *Osmunda regalis* when old may produce branches from the older meristematic region. The outgrowths of *Camptosorus* do not rightly belong to either of these groups. They were first described briefly by the writer in the Botanical Gazette of March, 1914. In old prothallia which have been subjected to long periods of drought, the older portions die away leaving groups of living marginal cells, or limited living areas below the margin. The older portions after they are no longer vegetatively active, conduct water to the active cell groups for a time. Some of the outgrowths are small, irregular, without apical cell or group and are but one cell in thickness. These bear rhizoids, and an abundance of antheridia but no archegonia. Others soon show apical groups, develop typical prothallial forms and produce rhizoids, meristematic cushions and both antheridia and archegonia. In short, these marginal proliferations show all the characteristics of normal prothallia, and after a time, through the breaking down of the tissue of the original plate, become entirely independent. Their sex organs are normal, and they produce sporophytes abundantly, sometimes while yet attached to the old prothallial mass. While they are found most abundantly on plants which have been subjected to unfavorable conditions, their formation is not wholly dependent upon such conditions, as evidenced by the fact that on some plants the marginal activity has proceeded far enough for independent growth by the time the first archegonia have died. The number of proliferations varies. Usually but two or three reach any considerable size, but in other cases as high as fifteen such independent growths have been counted. Fig. 38 shows a plant with rather unusual marginal development. In this figure *Q* shows the oldest portion of archegonial meristem, from which yet older dead tissue has broken away. In the irregular growth of the plant this cushion has taken a curved form, and its latest development is at *O* where a sporophyte has appeared. At points *A* to *R* marginal growth has produced proliferations. *P* was attached at point *T* and broken away in preparing the specimen for photographing. The dark central area and much of the lighter area around it are dead tissue. The root of a young sporophyte is shown at *S* and the leaf and stem buds at *O*. The first leaf was broken off in mounting. The mottled mass just behind the sporophyte at *O* shows the region of numerous antheridia and arche-

gonia. It must be borne in mind that these proliferations result from the activity of *groups* of marginal cells and not from the activity of single cells as in the formation of proliferous buds or gemmae, and that they do not appear as the result of mechanical injury to the parts producing them.

Summary of Camptosorus rhizophyllus.—The following freatues of the prothallial life of *Camptosorus* indicate to what extent the sexual generation of this fern is a factor in its distribution: Mature prothallia withstand practically unlimited interrupted drought as found in nature, if not exposed to direct sunlight, producing sporophytes through fertilization at the time of occasional showers.

The prothallia may survive exposure to experimental conditions of extreme desiccation for periods of four to six days.

A temperature of − 12° C. is fatal to most exposed plants.

Where winter exposure does not result fatally, the direct production of marginal proliferations may continue the prothallial life indefinitely.

The following facts concerning spore production and germination are also important factors: Spores are shed through a long period of time, some being retained in the sporangia until the spring following their formation, thus making possible sexual multiplication where the winters are too severe for the survival of the prothallia.

The spores germinate irregularly in point of time, thus offsetting the rather greater susceptibility of young prothallia to damage by drought.

ASPLENIUM PLATYNEURON Oakes.

In southern Indiana *Asplenium platyneuron* Oakes, is very common, growing at times on the sides of damp wooded ravines, where fronds 30–40 cm. in length are not rare, and occasionally in greatly reduced form on dry limestone cliffs and ledges. It is most abundant, however, in open woods along high ridges and on dry hillsides. The presence of many juvenile plants in the last named habitat has suggested the likelihood of conditions favorable for gametophyte development, and thus led to the present study. Most of the material for this work was taken from a steep slope of clay and light humus overlying limestone, with a southeast exposure. No springs are in evidence at this place and the hillside is but slightly covered with briers and bushes grown up since the timber was cut off three or four years ago.

In general the problem and means of attack are the same as in

the case of *Camptosorus*. The same culture methods have been used, and the same apparatus for securing extreme conditions. In fact cultures have been kept along beside those of *C. rhizophyllus*, and in the desiccation experiments tubes containing the two plants were attached in series so that they received exactly the same exposure. The important differences in treatment were in the experiments involving varying light conditions.

Fronds were collected June 24 and October 18, 1913. The latter collection was of fronds grown up after the rain of September 12. Cultures were prepared on soil, also on culture solutions as described in a later paragraph. The following features in the germination and development may be stated as generally true. The growth of the prothallia is much more uniform than in the case of *C. rhizophyllus*, although on soil cultures it is not unusual to find germinating spores and plants with twenty-celled plates side by side. The prothallia grow rapidly and symmetrically in either sunlight or full diffuse light. In twenty days after the spores are sown plants of five to fifteen cells may be found. At the end of ten weeks most of the plants show mature antheridia and archegonia. These points are true for cultures grown at an average temperature of 21° C. Only after the plants were sexually mature have they been subjected to the various conditions of experiment touching resistance to desiccation and low temperature.

Extreme Desiccation.—Plants were exposed to dry air in the desiccating apparatus with the following results:

I. Glycerine desiccator	all dead	after	6–7	days.
II. Sulphuric acid tube	"	"	"	6–7	"
III. Calcium chloride tube	"	"	"	5–6	"
IV. Freseniu -calcium chloride	"	"	"	5–6	"
V. Fresenius-sulphuric acid	"	"	"	5–6	"

The variable time of 5–6 and 6–7 days in these items is so placed because quite regularly a few plants of average size and normal appearance, have been found living after the shorter exposure. The writer is inclined to consider this a demonstration of remarkable individual variation in this respect comparable to that shown by the prothallia of this plant to a very unusual degree in other ways, to be noted later.

Extreme Natural Conditions.—Cultures allowed to become dry in the greenhouse showed no damage after three weeks, in diffuse light.

As an extreme trial a culture was watered October 8, 1913, and placed in full diffuse light with a temperature range of 12°–21° C., in air not in contact with water surface or sprays. On October 10 the soil surface appeared dry. Small portions were removed for revival at intervals until March 28, 1914. At that time, after 171 days without water, 20–25 per cent. of the plants were in condition for growth.

Cultures allowed to become air dry in sunlight at a temperature range of 13–25° C. during January, February and March, showed about 50 per cent. of the plants living at the end of four weeks, and showed a few living plants after six weeks of such exposure. Weather conditions make great variations in the results of these experiments, and the above data must be taken as the average of many repeated experiments. It has been very interesting to watch cultures of *A. platyneuron* and *C. rhizophyllus* exposed to the same light conditions. The greater resistance of the former to combined light and drought is very noticeable.

Winter Conditions.—Cultures placed outside on December 8, 1913, showed every plant living when brought inside on March 26, 1914. The lowest temperature to which these were exposed was − 12° C. and this seemed not to injure the plants in the least. This result was true with both dry and flooded cultures. Experiments to determine the minimum temperature limit have not been arranged, but field observations have indicated that a temperature of − 23° C. is not necessarily fatal.

Reaction to Special Light Conditions.—It has been stated above that the prothallia of *A. platyneuron* grow well in direct sunlight, other conditions being favorable, and that they are less liable to injury than those of *C. rhizophyllus* when cultures become dry in full light. It is now to be shown that they have a marked tendency to modify their growth as a result of variation in light intensity.

Spores and crushed sporangia were sown on a modified Knop's solution in 6 cm. petri dishes and stender dishes. Check cultures were made on distilled water and sterilized tap water. The Knop's solution was prepared as follows: A. KNO_3, 2 g.; $MgSO_4$, 2 g.; K_2HPO_4, 2 g.; Aq. dist., 2,000 cc. *B.* $Ca(NO_3)_2$, 4 g.; Aq. dist., 3,000 cc.

For use one part of *A* is added to three parts of *B* and boiled fifteen minutes. A series of check cultures was made with a solution similar to the above but with sterilized rain water instead of distilled water. No difference could be noted between the two series of cultures.

Variations of temperature between 13° and 25° C. affect development only as to rapidity. Cultures placed where they received equal light, but with a constant difference of 11° C. in temperature, develop similarly in every way, but those in the warmer place grow much more rapidly. This is true of plants in all culture media. Plants of cultures changed from a temperature of 13° C. to 24° C. show none of the peculiarities described below, as long as the light conditions remain the same.

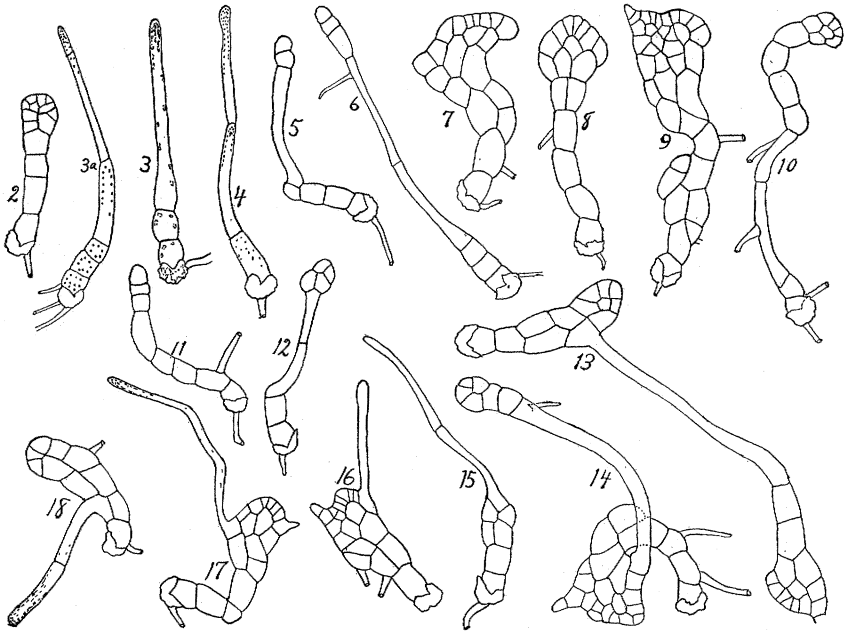
Spores may begin germination and produce one or two rhizoids when sown on distilled water, but will only very rarely show any cell division or chlorophyll formation. One series of such cultures was started August 30, 1913. After being subjected to a temperature of 16° C. and diffused light (.083) for seven weeks these cultures showed more than 90 per cent. of the spores with one or two rhizoids, varying in length from five to fifty times the greater spore diameter. At the end of this period nutrient solution was added and all the spores with rhizoids continued normal germination. Other cultures were allowed to wait ten to twelve weeks before receiving nutrient solution. Only about 50 per cent of the spores produced plants after the twelve week period.

Prothallia submerged in the culture solution tend to develop long protonemal chains of cells (fig. 20) and but rarely produce plates. The growth of submerged plants is also much slower than that of floating plants. Plants on the surface of the culture fluid develop in every way as plants on soil, but do not form antheridia or archegonia. At least, cultures kept under favorable conditions for eight to nine months show no sex organs. In this discussion floating plants are considered except where otherwise stated.

In the attempt to determine the influence of light of different intensities and of varying intensity, cultures with prothallia at various stages of development have been used. As a basis for comparison the history of one culture is here transcribed in detail from the record. The light values given were determined by means of a Solio photometer, and are based on 1. = full sun 1.00 P. M. of a clear day. Spores were sown on Knop's solution in a stender dish, October 13, 1913, and left in the greenhouse vestibule at an average temperature of 21° C. and with a maximum daily light value of .083.

October 28, nearly all the spores have germinated and most of the plants are composed of three to five plump cells in chains, with abundant chloroplasts. *Culture was placed under a saucer raised 1 cm. from the table, light value .011.*

November 4, culture shows chiefly 4–10 celled prothallia. Some plants show plates of 2–6 cells. Some distal cells are attenuated with the chloroplasts crowded in the extreme end (figs. 4, 23). For position of chloroplasts see also figs. 26*b*, 32, 33.



FIGS. 2–18. Camera lucida drawings of prothallia of *A. platyneuron* grown on Knop's solution. (× 240.) See text for description.

November 11. Plants show growth along three lines, increase in number of cells in plates or chains and increase in length of attenuated cells. Largest plants show 20 cells. *Culture removed to light of 0.8.*

November 17. Plants show rapid growth of plates with the beginning of apical groups. Attenuated filaments show normal plump cells at their distal ends, and in some cases, show divisions preparatory to plate formation (text-figs. 5, 6, 11, 12).

November 25. Plants show continued growth as last noted. Some show plates up to 40 cells where no attenuation had taken place, and up to 20 cells at the tips of attenuated cells. Some filamentous forms show branches (text-figs. 7, 8, 9, 10).

December 1. Continued growth as above. *Culture returned to reduced light, .011.*

December 17. Branches have grown from tips and sides, and rarely from apical cells, in every way like the attenuated growths referred to above (figs. 15, 16, 17, 18, 29, 32, 33).

December 29. The extensions have grown up to a maximum of three cells in length (figs. 25, 26). *Culture returned to strong light, o.8.*

January 5. Plants show normal beginnings of plates at the tips of second attenuations (figs. 13, 14, 19, 21, 22).

This culture was allowed to remain in full light. Each plate formed at the tip of an attenuated cell grew normally as an independent

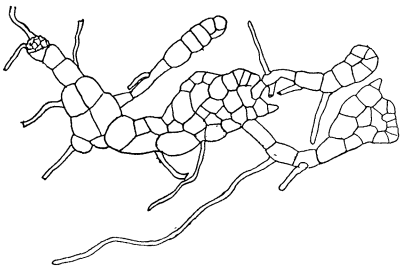


FIG. 19. Prothallium of *A. platyneuron* showing outgrowths from different parts to form independent plants, as a result of a period of reduced light followed by a period of stronger light. (× 52.) Traced from a photomicrograph.

plant. The primary plates also continued normal growth. In each citation given above the figures are from plants taken from one culture at one time, and usually from a single mass removed on a needle point.

The above account is that of a typical culture. There were variations in time and extent of reaction, due to weather conditions; but there was always a marked reaction in *some plants* and a seeming indifference *in others*. This individual difference is a point of special interest, manifested at every change in condition, and will be discussed at length.

Every culture subjected to variations of light intensity sufficient to produce changes in the manner of growth, has presented upon examination two groups of plants. The greater number of plants show reactions as described above. Some plants—about 25 per cent of each culture—on the other hand, show no noticeable reaction to light changes. In order to make sure that age had nothing to do with such variation, cultures were made by sowing spores on distilled water, and after three weeks when all viable spores had produced rhizoids,

adding Knop's solution. The resulting growth was very uniform in rate (fig. 24), and most of the plants showed the first division indicative of plate formation at the same time. But when such a culture was removed from full to reduced light, the results were by no means uniform. The larger number of plants show the formation of attenuated cells already described. But side by side with these forms are other plants showing no tendency toward such growth. Again the same situation is found in cultures subjected to two to four alternating changes as in fig. 31 *a–h*. Here some plants are shown continuing regular growth and plate formation without any sign of branching or attenuation. The growth, measured by the number of cells formed, is not quite as rapid under conditions of reduced light as in full light. It must be noted, however, that in plants grown from the first in light just too weak (.011) for normal development this difference does not appear (fig. 30). The sensitiveness to light variations is also shown by the following data. Cultures were grown continuously in light of maximum value of .025 and produced plants six to ten cells in length, slightly attenuated. From the middle of December up to the latter part of February there were alternating periods of three to ten days of very bright and very dark weather. During this time the cultures mentioned above showed the zones of attenuated cells and plates, just as produced by the more extreme changes of the experiments (fig. 27). This at least suggests that the changes in growth are due to the *variation* in light intensity and not to its absolute value. This view is further substantiated by the fact that in a few cases plants grown in light of maximum value of .025 and then brought into light of .8 maximum value produced the outgrowths regularly produced after a change from strong light to weaker light. It seems from this that the variation in light intensity is only a stimulus to a change in growth, and does not determine the nature of that change. Finally it must be noted that as a result of changes of light intensity some plants tend to branch rather than produce attenuated cells. Extreme cases are much like that in fig. 20. A younger and more moderate case is shown in fig. 28. Such plants finally produce several apical groups and plates (fig. 34).

As has already been suggested there is much difference in the origin of the branches and attenuations which follow the change in light intensity. In a small number of cases the apical cell produces the outgrowth (figs. 25, 26*a*, 32). But more often such growth does not

take place from the apical cell or any of the neighboring cells, but from older cells in regions of reduced activity (figs. 16, 17, 18, 19, 21, 22, 26*b*, 29). When plants with plates 1–2 mm. wide are subjected to reduced light the branches or attenuated cells are produced by any part of the margin, from the original protonemal cells up to the apical group, and even at times by included cells growing out perpendicular to the surface of the plate. In other cases the whole apical group is pushed out by a band of slightly elongated cells until a distinct pluricellular branch is formed (fig. 35). To make sure that these results were not unduly influenced by the fact that the plants were grown on culture fluids, regular soil cultures were subjected to the same conditions. The results were the same with the exception that, in the case of plants with well developed plates .5 mm. or more in width, the production of broad outgrowths like fig. 35 was rather more usual than with the plants of water cultures.

Summary of Reaction to Light.—The results of experimental work relative to reaction to light stimulus may be summed up as follows: Plants develop normally in almost identical manner under approximately constant light intensities within a wide range.

Most of the plants respond to a sharp reduction of light by producing elongated cells or pluricellular branches.

Some plants show a similar reaction when the light intensity is increased.

Some plants do not respond to variations in light intensity by any peculiarity of growth.

It seems that the *change* in light intensity stimulates unusual growth in most of the plants, but the plants show a marked individual variation in their response to that stimulus.

Field Work.—The extreme conditions of the summer of 1913 have already been given. To show that the *Asplenium platyneuron* fields under observation received the full force of these conditions it need only be said that many clumps of mature sporophytes were killed outright, after maturing a few spores in the early summer. On December 3, 1913, a careful search was made for prothallia in the field. Numerous well developed plants were found on the soil, limestone fragments and dead twigs or leaves about the old sporophyte clumps. Most of these ranged from ten to fifteen cells up to those maturing their first antheridia, and averaged a little smaller than the plants of greenhouse cultures from spores sown September 4, 1913. A second

group of prothallia averaged 2–4 mm. wide and showed earlier dead tissue and antheridia and archegonia long past mature (figs. 36, 37). The younger portions of these plants bore mature sex organs and in a few cases sporophytes of one or two leaves. The similarity of the younger plants in size and development to those in cultures from spores collected and sown September 4, just before the rain of September 12 first made possible germination and growth in the field, leaves little room for doubting that these plants were produced by the germination of spores in the fall of 1913. The plants of the second group were much larger than those of cultures started August 30. In fact they were quite similar in every way to plants examined in April, 1914, of cultures started in September 1913 and allowed to remain out of doors during most of the winter of 1913–14. Their age is evidenced by the dead basal areas. There are two possible explanations of the appearance of these plants. The first, that the plants grew from spores which had lain dormant through the winter and had germinated in the early spring of 1913, need hardly be considered, because no indications of a tendency to lie dormant has been found in experimental cultures, and because the growing season in the spring of 1913 was too brief for the production of plants of the size found. The second possibility, and what seems to the writer the only plausible explanation, is that the larger plants were produced by the germination of spores in the autumn of 1912. This view includes the belief that the plants had survived the winter of 1912–13 with a minimum temperature of − 23° C. in the field. The experimental data presented above have prepared the way for such a belief, and the finding of many living plants in late January, 1914, and even in April, makes the possibility of surviving the winter not only a belief but a certainty. The writer feels quite safe in saying that a considerable portion of the prothallia of this fern, grown from spores germinating in the late summer and autumn, live through the winter and produce sporophytes the next summer.

The following facts touching the reaction to light have been noted in the field. The usual place of growth of prothallia is under old leaves in crevices in limestone or in depressions in the soil. Any chance breeze may by shifting the covering produce variations in light intensity equal to the widest range in the writer's experiments. In fact nearly all the facts of behavior noted in the experimental cultures have been verified in the field by the finding of forms from the branch-

ing protonemal type up to independent secondary prothallia produced by outgrowths from old plants to which in some cases they were yet attached. That specimens found in the field are *A. platyneuron* cannot be determined with absolute certainty. The absence of other ferns in the immediate neighborhood and the abundance of prothallia close around old clumps of sporophytes leaves little doubt on this question. Careful comparison of plants from the field with those of cultures, including measurements of cells, have been made, however, and in some cases sporophytes large enough to be recognized as juvenile forms of *A. platyneuron* have made the identification more certain.

Summary of Asplenium platyneuron.—The facts of the growth and development of the prothallia of *A. platyneuron* of ecological importance may be summed up as follows: There is a variation of a few weeks in the time required for the germination of spores.

The prothallia in experimental cultures withstand a temperature of − 12° C. without injury, and in the field they have survived exposure to a temperature of −- 23° C. and later produced sporophytes.

The resistance to extreme desiccation, brought about artificially, is slightly lower than in the case of *Camptosorus rhizophyllus*, but the resistance to extreme natural drought is much more marked than in that fern.

Mature prothallia are uninjured by exposure to repeated periods of three to four weeks of drought as met with in nature.

Extreme sensitiveness to changes in light intensity leads to the production of protonemal branched prothallia, or attenuated outgrowths, either of which may lead to a vegetative increase of the gametophyte and the formation of independent plants.

The prothallia show marked individual differences in reaction to light changes and in the power to survive exposure to conditions of extreme desiccation.

The author wishes to acknowledge his indebtedness to Prof. D. M. Mottier and Dr. F. M. Andrews, of the Botany Department of Indiana University, for their kind encouragement and valuable direction and help in the present work.

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DESCRIPTIONS OF PLATES XLIX AND L

PLATE XLIX

With the exception of figs. 27 and 34 these photomicrographs were made with Zeiss 16 mm. apo. obj. and No. 4 comp. oc. The others were made with Leitz No. 1 achromatic obj. and oc. No. 2. Cramer's "slow Iso" plates were used.

Asplenium platyneuron prothallia

FIGS. 20–22. Plants grown in full light, subjected to reduced light for sixteen days and then returned to full light. (× 75.)

FIG. 23. Young plants subjected to reduced light. See fig. 3, 3*a*, 4. (× 75.)

FIG. 24. Group of plants from culture of spores allowed to remain three weeks on distilled water in good light before Knop's solution was added. Photo taken ten days after the addition of nutrient solution. (× 75.)

FIGS. 25, 26. Plants after a second change to reduced light. (× 75.)

FIG. 27. Plants from a culture grown in weak light, showing zones of varying growth corresponding to periods of bright and dark weather. (× 40.)

FIG. 28. Branched protonemal form. (× 75.)

FIG. 29. Plants subjected to a second period of reduced light. This with 21, 22, and 26*b* shows attenuated outgrowths from older portions. (× 75.)

FIG. 30. Plants showing uniform growth in light just too weak for normal development. (× 75.)

FIG. 31 *a–h*. A group of plants from one culture, showing the great difference in response to variations of light intensity. (× 75.)

FIGS. 32, 33. Plants grown in full light and then placed in reduced light, showing attenuated branches from cells near the apical group. (× 150 and 75.)

FIG. 34. Final formation of several plates by a branched form. Two other plates were attached at *a* and *b* but were torn away in mounting. (× 22.)

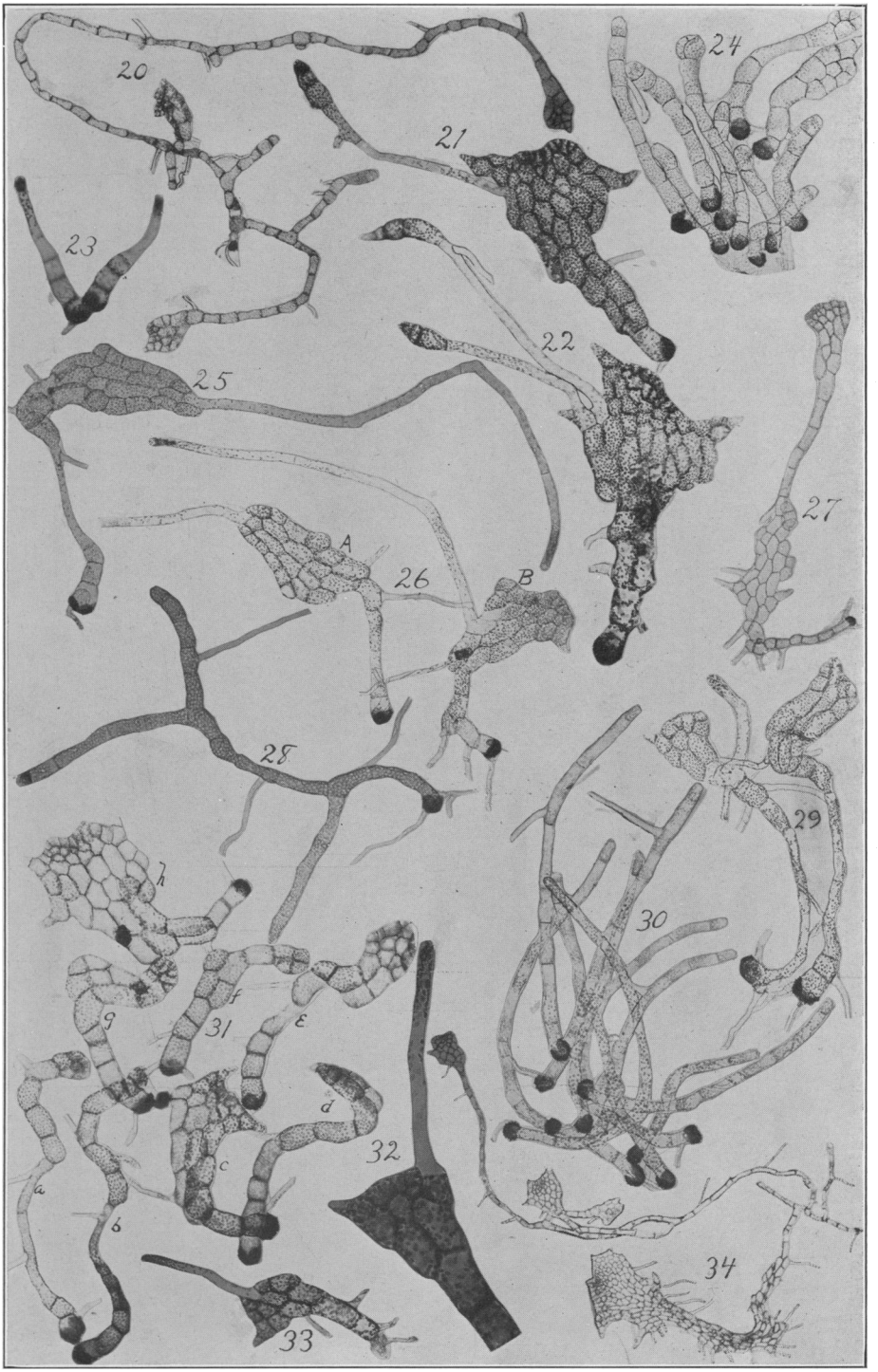
PLATE L

Photomicrographs made with Leitz achromatic obj. No. 1 and oc. No. 2.

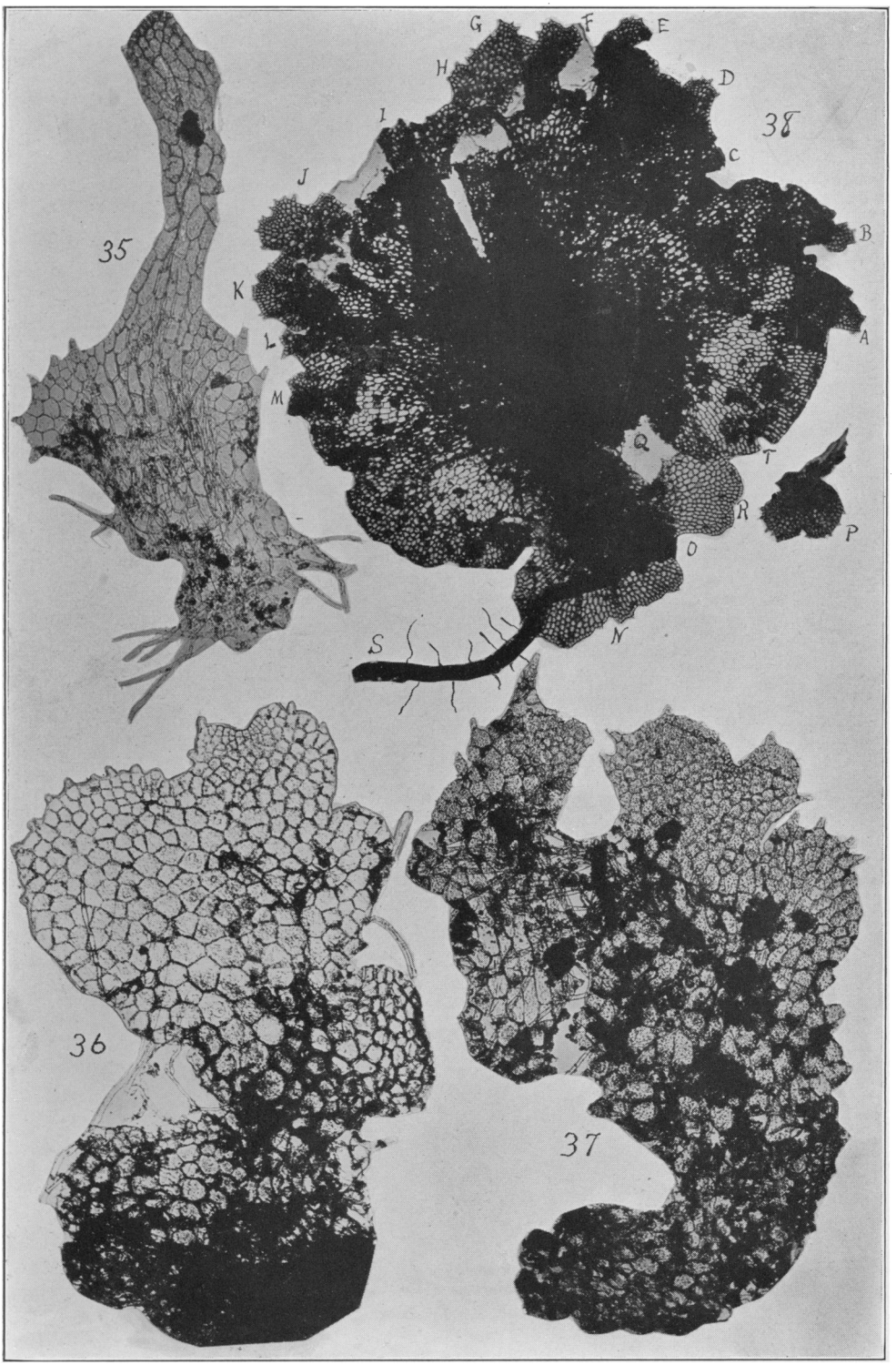
FIG. 35. *Asplenium platyneuron*. A plant showing a pluricellular branch, a common form among mature plants of soil cultures changed from strong to reduced light. (× 52.)

FIGS. 36, 37. *A. platyneuron.* Plants collected in the field, probably more than twelve months old. Much of the old tissue was torn away in cleaning and mounting the specimens. (× 52.)

FIG. 38. *Camptosorus rhizophyllus.* Plant from a culture thirteen months old. *A–R*, regions of marginal growth. *P*, an independent secondary prothallium, formerly attached at *T*. *S*, root of a young sporophyte. *Q*, oldest portion of the archegonial cushion. See text for full description. (× 22.5.)



PICKETT: PROTHALLIA OF CAMPTOSORUS AND ASPLENIUM.



PICKETT: PROTHALLIA OF CAMPTOSORUS AND ASPLENIUM.